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Lipid Apheresis: An In Vivo Application of Plasma Delipidation With Organic Solvents Resulting in Acute Transient Reduction of Circulating Plasma Lipids in Animals

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Despite primary and secondary prevention of coronary disease with lowering plasma cholesterol by diet and drug therapy, coronary heart disease remains the major cause of death in Western countries. Low density lipoprotein apheresis had the potential to make a significant impact as it acutely leads to a marked reduction in plasma cholesterol. However, recent preliminary results suggest that low density lipoprotein apheresis may not be more effective in preventing progression of coronary disease than current drug therapy. We have devised a new technique, termed lipid apheresis, which removes cholesterol and triglycerides from plasma but retains the apolipoproteins. This procedure shows great promise in stimulating regression beyond current therapy. Lipid apheresis, a new extracorporeal procedure based on plasma delipidation with the organic solvent mixture butanol-diisopropyl ether, was applied to hypercholesterolemic and normocholesterolemic roosters. Approximately 25% of the calculated blood volume was removed from the animals. The plasma was separated from the blood cells. The plasma was delipidated for 20 min with the organic solvent mixture. The delipidated plasma containing all proteins, including the apolipoproteins and other ionic constituents, was remixed with the blood cells and infused back into the identical donor animals. Analyses of serial blood samples collected from lipid apheresed and sham treated animals up to 16 h after infusion revealed that lipid apheresis caused acute, marked reductions in plasma lipids. The pattern and extent of the plasma levels of cholesterol were different in the hypercholesterolemic animals when compared with normocholesterolemic animals, indicating that a readily extraplasma cholesterol pool in the hypercholesterolemic animals was rapidly mobilized into the plasma pool. Haematological and biochemical parameters in the blood of the treated animals were unaffected. These observations may have important implications for the management of human atherosclerosis.

Key words: extracorporeal, sham treatment, atherosclerosis, hypercholesterolemia

INTRODUCTION

It is now generally accepted that an elevated plasma cholesterol concentration is causally related to atherosclerosis [1]. The major atherogenic lipoprotein is low density lipoprotein (LDL). The precise mechanism by which elevated levels of LDL-cholesterol results in atherosclerosis is not entirely clear. Despite progress in the achievement of lowering plasma cholesterol to prevent coronary heart disease by diet and drug therapies, surgical revascularization procedures, angioplasty, and LDL apheresis, this disease remains the major cause of death in Western countries [2]. A recent study comparing LDL apheresis plus simvastatin and a group on simvastatin only showed no difference in progression or regression of coronary atherosclerosis or in clinical events [3]. Serial angiography in coronary arteries provides validated surrogate endpoints for large clinical trials [4]. Therefore, these preliminary data [3] suggest that LDL apheresis may not have any advantage over current drug therapy.

The importance of apolipoproteins in lipid metabolism is well recognized [5]. In order to investigate the transport of cholesterol from peripheral tissues to the liver for clearance from the body ("reverse cholesterol transport") [6], researchers have employed systems in which the unidirectional efflux of cholesterol from cultured cells

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Abbreviations: CA, cholesterol apheresis; DEEE, diethyl ether; DIPE, diisopropyl ether; HDL, high density lipoprotein; LA, lipid apheresis; LDL, low density lipoprotein.

could be monitored. Since the putative acceptor of cholesterol in reverse cholesterol transport is high density lipoprotein (HDL), there has been interest in investigating the role of the apolipoproteins of HDL in cell cholesterol efflux [7–9].

It has previously been reported from this laboratory [10,11] that delipidation of plasma with a mixture of butanol and diisopropyl ether (DIPE) resulted in the removal of lipids from plasma. With this procedure, the apolipoproteins remained soluble in the plasma. Furthermore, delipidation of plasma did not affect the concentrations of the ionic constituents, proteins, pH, and enzyme activities [10,11]. Other lipid-associated proteins in biological fluids are also unaffected when their lipids are removed by the delipidation procedure [12–15]. Removal of lipids from such proteins does not affect their physiological role [16,17].

The plasma, once delipidated, is capable of recombining with extracellular lipid [18].

HDL stimulates excretion of excess intracellular cholesterol from cells, presumably by interacting with a cell-surface receptor [19]. Plasma delipidation converts the spherical HDL particles to disc or amorphous forms but this does not alter receptor binding activity [20]. It is known that disc and amorphous forms of HDL (cholesterol-depleted) are better substrates for removing cholesterol and are more effective in "reverse cholesterol transport" than is native HDL. It has been shown that apo-HDL, which is present in delipidated plasma, can remove cholesterol from cells grown in culture. Moreover, delipidated HDL is a much better (more than 6-fold) cholesterol acceptor from cells than is native HDL [21]. Recently it has been shown that discoidal HDL inhibits acetylated LDL uptake by macrophages, probably by phospholipid transfer from HDL to LDL [22].

Cell receptors recognize LDL even if the LDL contains virtually no cholesterol as derived by this delipidation procedure [20]. Hence the supply of cholesterol to cells can be reduced by exposing such cells to delipidated LDL in the delipidated plasma instead of native LDL.

The combination of the reduction of supply of LDL-cholesterol (delipidated LDL) to cells *and* the enhanced removal of cholesterol from peripheral cells by delipidated HDL (increase reverse cholesterol transport) both show enough promise to investigate whether this plasma delipidation approach may be of benefit as a treatment for atherosclerosis in an extracorporeal system.

We now describe for the first time the application of the delipidation procedure of Cham and Knowles [10] for the extracorporeal elimination of lipid but not apolipoproteins from plasma of roosters by a specific mixture of organic solvents, butanol and DIPE. We have termed this procedure lipid apheresis (LA) when applied for the removal of lipids and cholesterol apheresis (CA) when applied specifically for the removal of cholesterol from plasma.

MATERIALS AND METHODS

Animals

The roosters used in this study were of White Leghorn Hiline strain and were obtained as 1-day-old chicks. All roosters from 8 weeks of age were transferred into individual cages. Water and feed were supplied unrestricted.

At 8 weeks of age, ten control birds were fed a commercial poultry ration for 31 days and another group of ten birds were fed on the same diet, which was supplemented with 2.6% cholesterol for a period of 31 days.

Seven animals of each group were then subjected to LA. Three animals of each group had sham treatments.

All 20 animals were kept off their feed for 3 h following reinfusion of their autologous blood. The care of the animals was in accordance with the ethical standards of the University of Queensland.

Lipid-Apheresis Procedure

Approximately 25% of the calculated blood volume was collected from a brachial vein of the animal with a 21 gauge needle and syringe. The total blood volume was estimated as 8 percent of the body weight [23]. The blood was collected in heparinized tubes and immediately centrifuged at 900*g* for 5 min at room temperature. The plasma was separated from the blood cells. The blood cells were kept in the tubes. The plasma was delipidated for 20 min with a mixture of butanol-DIPE, 25:75 (v/v) in a ratio of one volume of plasma to two volumes of butanol-DIPE mixture (organic phase) [18]. After extraction the mixture was centrifuged at 900*g* for 2 min to separate the plasma and organic phases. The organic phase (upper layer) was removed, free of plasma phase, by careful aspiration with a pasteur pipette under vacuum. Traces of butanol in the plasma phase was washed out with two volumes of diethyl ether (DEE) for 2 min by end-over-end rotation at 30 rpm. The mixture was then centrifuged at 900*g* for 2 min to separate the plasma and ether phases. The ether phase was subsequently removed by aspiration with a pasteur pipette. Residual ether was removed by evacuation with a water pump aspirator at 37°C. This procedure yielded delipidated plasma. The delipidated plasma was remixed with the original blood cells which was then reinfused through a brachial vein back into the donor animals. The duration of the entire procedure, that is, removal of blood from the animal to reinfusion of treated blood back to the animal, was approximately 1 h (range 50–70 min).

Sham Treatment Procedure

This was essentially the same as the LA procedure with the exception of plasma delipidation with the organic solvents. The blood was collected in heparinized tubes and immediately centrifuged at 900*g* for 5 min. The plasma was separated from the blood cells. The blood cells and plasma were kept in separate tubes for approxi-

mately 45 min. The plasma was remixed with the original blood cells which was then reinfused through a brachial vein back into the donor animals.

Laboratory Measurements

Various clinical chemical and haematological parameters were measured using standard techniques in the Departments of Clinical Chemistry and Haematology at the Royal Brisbane Hospital in Brisbane. The covariant of analysis for the measurement of the parameters is less than 2 percent. Plasma total lipids were measured colorimetrically using the Boehringer Mannheim kit which is based on the procedure of Zollner and Kirsch [24]. Measurements of plasma levels of cholesterol, triglyceride, and choline-containing phospholipids were determined by enzymatic methods [25,26] using reagents from Boehringer Mannheim and a Cobas-Bio centrifugal analyzer (Hoffman-La Roche, Zurich).

DEE, n-butanol, and DIPE in plasma were determined by gas chromatography at the Government Chemical Laboratories in Brisbane. The method is essentially the same as for ethanol determination in blood and is derived from the work of Cooper [27]. A Hewlett-Packard 5790A Gas Chromatograph was used with a 1.8 m Carbapak column and flame ionisation detector. The column temperature was maintained at 120°C; detector temperature was 200°C; and the carrier gas flow-rate, 50 ml/min. The run time for a single analysis was 6 min. The order of elution and retention time in minutes was DEE, 1.18; isobutanol (internal standard), 2.08; n-butanol, 3.25; and DIPE, 4.55. The detection limit in plasma for each solvent was 0.05 mg per ml of plasma.

Statistical Analyses

Comparisons between the various times after reintroduction of delipidated autologous blood were made with respect to the percentage change from baseline for the following variables: cholesterol, triglycerides, phospholipids, and total lipids.

Within-group comparisons were made using Wilcoxon's signed-rank test. Adjusted mean percentage changes were obtained using an analysis of covariance on the individual percentage changes with treatment as a model effect and baseline value as a covariate. Adjusted mean percentage changes were similar to the equivalent unadjusted values and therefore the difference in unadjusted mean percentage changes was considered an appropriate estimate of the difference between baseline value and treated value.

The comparability of the treatment groups of baseline and post-infusion levels of delipidated autologous blood resulting in the reduction of plasma lipids was assessed by means of analysis of variance on the ranked values of the reduction of plasma lipid levels with delipidated blood infusion as a factor.

TABLE I. Effect of Normal and High Cholesterol Diets on Plasma Lipid Levels and Body Weights†

Components	Control diet (n = 10)	High cholesterol diet (n = 10)
Cholesterol (mmol/L)	3.14 ± 0.72*	11.43 ± 3.67*
Triglyceride (mmol/L)	0.70 ± 0.23**	1.26 ± 0.57**
Phospholipid (g/L)	2.17 ± 0.26	2.19 ± 0.49
Total lipid (g/L)	8.41 ± 2.50*	18.92 ± 5.35*
Body weight (kg)	2.03 ± 0.18	2.06 ± 0.15

†The body weight, plasma concentrations of cholesterol, triglyceride, phospholipid, and total lipid of roosters that had been on a control or hypercholesterolemic diet for 31 days are shown. Data represent mean ± SD.
*$P \leq 0.0005$.
**$P \leq 0.01$.

Two-sided tests were used for all statistical comparisons of LA treatment. All probability values were rounded to two decimal places and the statistical significance of LA treatment or dietary treatment comparisons was declared if the rounded probability value *(P)* was less than or equal to 0.05.

RESULTS

Effect of High Cholesterol Diet on Plasma Lipid Levels

Roosters fed on a high cholesterol diet for 31 days became hyperlipidemic. Hyperlipidemia was due mainly to hypercholesterolemia. The plasma cholesterol, triglyceride, and total lipid concentrations increased significantly. Plasma phospholipid concentration was unaffected by the high cholesterol diet. There were no significant differences in body weight between the hypercholesterolemic and normocholesterolemic animals (Table I).

In Vitro Effect of the Delipidation Procedure on Plasma Components

Delipidation of plasma in vitro did not affect the concentrations of total protein, albumin, immunoglobulins, and other ionic and chemical constituents. The activities of AP, LDH, AST, GGT, and ALT were also unaffected; only lipids were removed from the plasma by the delipidation procedure. Table II details the concentration or activities of these plasma components of a representative sample of plasma from roosters before and after delipidation.

Effect of Infused Delipidated Autologous Plasma (LA) on In Vivo Plasma Lipids Over a Period of 16 h

Infusion of delipidated autologous plasma (approximately 25% of plasma volume) mixed with red blood cells (RBC) resulted in rapid reduction of plasma cholesterol (Fig. 1A), triglyceride (Fig. 2A), phospholipid (Fig. 3A), and total lipid (Fig. 4) concentrations. In the normo-

TABLE II. Quantitative Comparison of In Vitro Plasma Constituents Before and After LA of Rooster Plasma*

Component	Dimension	Before LA	After LA
Sodium ion	mmol/L	156	155
Potassium ion	mmol/L	3.9	3.8
Chloride ion	mmol/L	122	122
Total CO_2	mmol/L	24	22
Creatinine	mg/100 ml	0.03	0.03
Uric acid	mg/100 ml	0.57	0.59
Urea nitrogen	mg/100 ml	<1	<1
Inorganic phosphate	mg/100 ml	1.53	1.57
Calcium	mg/100 ml	2.83	2.90
Total cholesterol	mmol/L	3.60	0
Triglyceride	mmol/L	0.93	0
Phospholipid	g/L	2.72	0
Total lipid	g/L	8.21	0
LDH	IU/L	519	521
AST	IU/L	235	233
GGT	IU/L	20	20
ALT	IU/L	<5	<5
AP	IU/L	1,310	1,325
Glucose	mg/100 ml	15.5	15.7
Globulins	g/L	34	34
Albumin	g/L	33	33
Total protein	g/L	67	67

*These findings are representative of the results obtained from four experiments. Only the lipid parameters were significantly different before and after in vitro LA.

lipidemic animals the reduction of these plasma lipids was immediate and was sustained for up to 150 min. Sixteen hours after infusion of delipidated autologous plasma, the concentrations of these lipids had returned to the pretreatment values. In contrast, with the hyperlipidemic animals, infusion with delipidated autologous plasma reduced plasma levels of cholesterol, total lipids, and phospholipids only transiently. Fifteen minutes after infusion of delipidated autologous plasma these lipids were significantly reduced in plasma within the animals. Thirty minutes and thereafter, following infusion of delipidated autologous plasma, these plasma lipids were generally lower in concentration than the pretreatment baseline concentrations. However, because of the high standard deviations these values were not significantly different from the pretreatment values. The response patterns of plasma triglyceride concentration to infusion of delipidated plasma were similar in the normolipidemic and hyperlipidemic animals.

Effect of Sham Treatment on In Vivo Plasma Lipids Over a Period of 16 h

Reintroduction by infusion of autologous plasma (approximately 25% of plasma volume) remixed with RBC did not result in significant changes in plasma cholesterol (Fig. 1B) concentrations in the normolipidemic and in hyperlipidemic animals throughout the 16h experimental period. The sham treatment resulted in reduction in plasma triglyceride (Fig. 2B) concentrations in the normolipidemic and hyperlipidemic animals. Plasma phospholipid concentration in the normolipidemic animals did not change significantly, whereas in the hyperlipidemic animals significant changes were induced by the sham treatment (Fig. 3B).

Effect of Infusion of Delipidated Autologous Blood (LA) and Sham Treated Blood on In Vivo Haematological and Biochemical Parameters

Biochemical and haematological parameters obtained from each animal prior to and after infusion of delipidated autologous plasma and sham treated plasma remixed with the original blood cells indicated that these parameters remained within the recommended normal range. There were no significant changes in these parameters as a consequence of LA or sham treatment. Tables III and IV show the concentrations of various parameters in blood of representative animals before and 150 min after LA and sham treatment in normolipidemic and hyperlipidemic animals.

Residual Solvents in Plasma After LA

No solvents were detectable in plasma after LA. The detection limit was 0.05 mg solvent per ml plasma (0.005% w/v).

DISCUSSION

Plasma exchange and, more recently, selective LDL-apheresis are being increasingly used for the treatment of severe hypercholesterolemia. It has been reported that progression of coronary atherosclerosis can be stopped and sometimes regression can be achieved in humans [28]. LDL-apheresis permits a "selective" removal of the plasma LDL-cholesterol constituent. Depending on the procedure used, other plasma constituents, including HDL, may be removed [28]. However, all procedures remove whole lipoprotein complexes; that is, the lipid as well as the protein moieties. In such systems 40–80% decreases in plasma LDL cholesterol concentrations have been reported. The recovery of plasma LDL-cholesterol is approximately 7 days [29], which is consistent with de novo synthesis of lipoprotein particles. In contrast, this study shows delipidation of plasma removes lipids only. Using this procedure it has been shown that the apolipoproteins remain in the plasma [10,11,18,20,30–37]. When the delipidated autologous blood is infused into the normocholesterolemic animal (LA), the plasma cholesterol remains significantly lower for 2.5 h. Sixteen hours after infusion of the delipidated blood, plasma cholesterol concentration returned to the pretreatment value. It is of utmost importance to realize that in the hypercholesterolemic animals, plasma cholesterol concentration was significantly reduced only 15 min after infusion of delip-

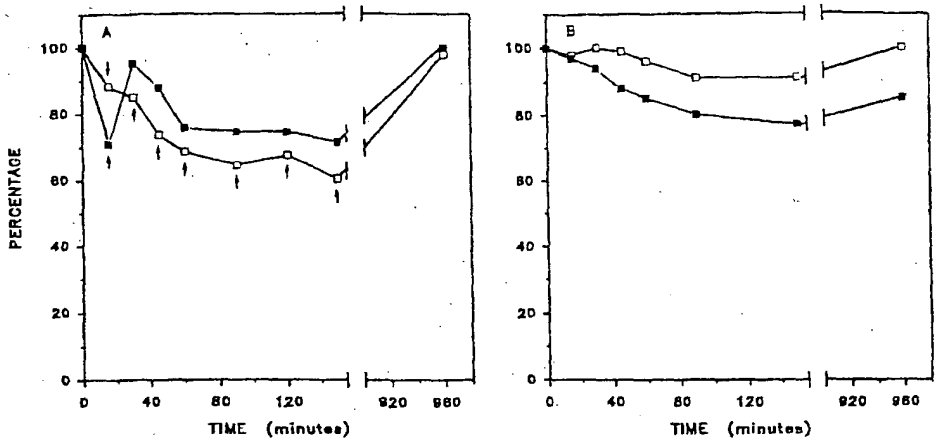

Fig. 1. Changes of plasma total cholesterol concentrations after lipid apheresis or sham treatment. The plasma cholesterol concentrations of roosters on control diet (□—□) and hypercholesterolemic diet (■—■) were determined before and at various times after reinfusion of lipid apheresed blood or sham treated blood. The results are expressed as percent changes. The data of the two groups of animals were pooled to determine statistical differences. The concentrations of plasma cholesterol before lipid apheresis (A) were 3.25 ± 0.87 (n = 7) and 14.10 ± 4.30 (n = 7) mmol/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. The concentrations of plasma cholesterol before sham treatment (B) were 3.18 ± 0.66 (n = 3) and 11.60 ± 2.53 (n = 3) mmol/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. Arrows indicate significant differences ($P < 0.05$) of those values compared to the pre-treatment values (100%).

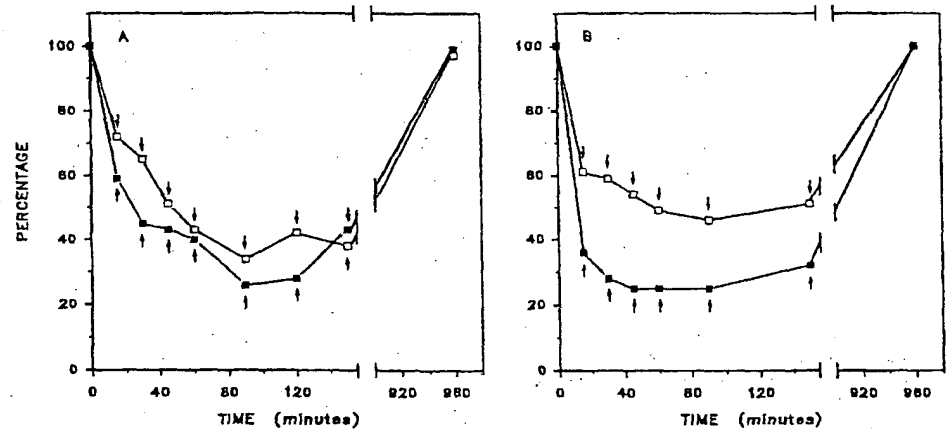

Fig. 2. Changes of plasma triglyceride concentrations after lipid apheresis or sham treatment. The plasma triglyceride concentrations of roosters on control diet (□—□) and hypercholesterolemic diet (■—■) were determined before and various times after reinfusion of lipid apheresed blood or sham treated blood. The results are expressed as percent changes. The data of the two groups of animals were pooled to determine statistical differences. The concentrations of triglyceride before lipid apheresis (A) were 1.05 ± 0.61 (n = 7) and 2.13 ± 1.17 (n = 7) mmol/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. The concentrations of triglyceride before sham treatment (B) were 0.65 ± 0.13 (n = 3) and 0.95 ± 0.33 (n = 3) mmol/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. Arrows indicate significant differences ($P < 0.05$) of those values compared to the pre-treatment values.

idated autologous blood. Thirty minutes after infusion, the plasma cholesterol concentration had returned to the pretreatment concentration and was not significantly different from the pretreatment values thereafter. These data strongly suggest that the reason for the different response observed in hypercholesterolemic animals when compared with normocholesterolemic animals is that more of an existing cholesterol pool in the hypercholesterolemic animals is rapidly mobilizible by the apheresis treatment.

It may be that the apolipoproteins in the delipidated blood interact and solubilize such cholesterol pools to produce newly formed lipid-protein complexes. Indeed, other work (submitted) indicates that "newly" formed lipoprotein complexes are present in plasma soon after

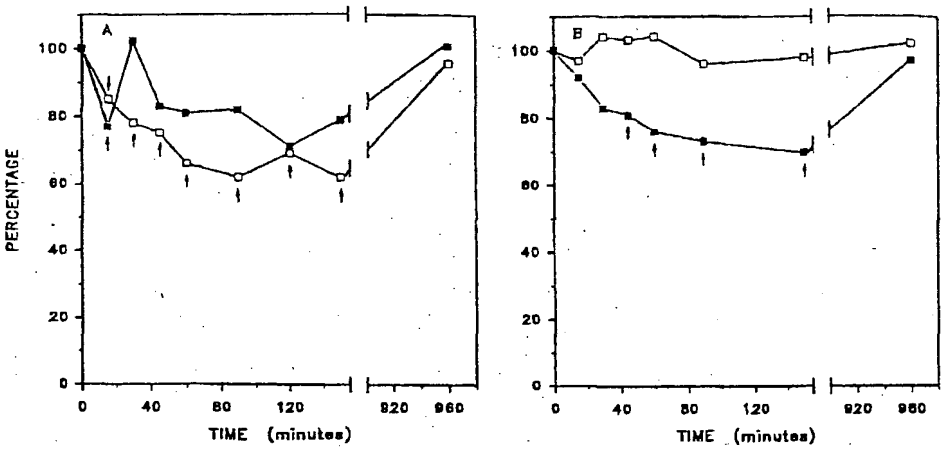

Fig. 3. Changes of plasma phospholipid concentrations after lipid apheresis or sham treatment. The plasma phospholipid concentrations of roosters on control diet (□—□) and hypercholesterolemic diet (■—■) were determined before and eight times after reinfusion of lipid apheresed blood or sham treated blood. The results are expressed as percent changes. The data of the two groups of animals were pooled to determine statistical differences. The concentrations of plasma phospholipid before lipid apheresis (A) were 2.73 ± 0.67 (n = 7) and 2.45 ± 0.52 (n = 7) g/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. The concentrations of plasma phospholipid before sham treatment (B) were 1.90 ± 0.13 (n = 3) and 1.90 ± 0.59 (n = 3) g/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. Arrows indicate significant differences ($P < 0.05$) of those values compared to the pre-treatment values (100%).

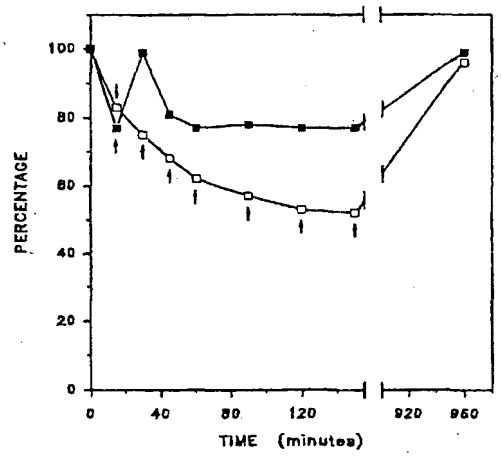

Fig. 4. Changes of plasma total lipid concentrations after lipid apheresis. The plasma total lipid concentrations of roosters on control diet (□—□) and hypercholesterolemic diet (■—■) were determined before and eight times after reinfusion of lipid apheresed blood. The results are expressed as percent changes. The data of the two groups of animals were pooled to determine statistical differences. The concentrations of plasma total lipids before lipid apheresis were 8.41 ± 2.50 (n = 7) and 18.92 ± 5.35 (n = 7) g/L for the normocholesterolemic and hypercholesterolemic roosters, respectively. Arrows indicate significant differences ($P < 0.05$) of those values compared to the pre-lipid apheresis values (100%).

infusion of delipidated blood. One of these lipoprotein fractions behaves like the recently described pre-beta HDL [38,39]. The Apo A-I, which is present in delipidated plasma [10,11,29,31], may stimulate net efflux of cholesterol and phospholipid from cells [21,40] and therefore plays a role in reverse cholesterol transport and the prevention of atherosclerosis [41]. The rapid recovery of plasma cholesterol concentration suggests that de novo synthesis of the lipoprotein particle was not occurring, but that the apolipoproteins were acquiring cholesterol from extraplasma pool stores. This is in direct contrast to observations with LDL apheresis.

There were no significant differences in the response of plasma triglyceride concentration after reinfusion of delipidated plasma when the normocholesterolemic animals were compared with the hypercholesterolemic animals. It is interesting that in general, the plasma triglyceride concentrations continued to drop up to 2.5 h after infusion of delipidated plasma. The results obtained with the sham treatment show that these observations are not due to lipid apheresis. The reason for the continuous drop in plasma triglyceride, phospholipid, and, to a lesser extent, cholesterol concentrations after sham treatment is unknown at this stage. However, the changes may be related to the heparin that was used as the anticoagulant with the blood collection and subsequently infused after treatment of the blood. Heparin is known to activate lipoprotein lipase.

Roosters fed on a diet containing 2.6% cholesterol became hypercholesterolemic. This is in agreement with previously reported studies [42].

Quantitative comparison of in vitro plasma constituents before and after delipidation of rooster plasma re-

TABLE III. Quantitative Comparison of Plasma Biochemical Constituents Before and 2.5 h After Reinjection of Approximately 25% of Delipidated Plasma Remixed With Original Blood Cells and Sham Treated Blood in a Normolipidemic and a Hyperlipidemic Rooster*

		Normolipidemic rooster				Hyperlipidemic rooster			
		Lipid apheresis		Sham treatment		Lipid apheresis		Sham treatment	
Component	Dimension	Before	2.5 h after	Before	2.5 h after	Before	2.5 h after	Before	2.5 h after
Sodium ion	mmol/L	157	150	141	144	124	138	140	136
Potassium ion	mmol/L	4.0	4.2	3.9	3.9	3.7	3.7	3.9	4.0
Chloride ion	mmol/L	119	121	104	106	99	109	102	101
Total CO_2	mmol/L	20	22	20	18	19	16	20	19
Creatinine	mg/100 ml	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.04
Uric acid	mg/100 ml	0.47	0.39	0.37	0.39	0.26	0.35	0.37	0.39
Urea nitrogen	mg/100 ml	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Inorganic phosphate	mg/100 ml	0.89	1.01	0.91	0.93	0.95	1.10	0.96	0.93
Calcium	mg/100 ml	3.27	2.92	3.20	3.30	2.05	3.95	3.30	3.18
LDH	IU/L	720	589	630	610	368	366	490	510
AST	IU/L	210	240	207	220	181	197	198	196
GGT	IU/L	22	16	<5	<5	<5	<5	<5	<5
ALT	IU/L	<5	<5	17	10	10	17	14	13
AP	IU/L	1,020	898	327	410	275	328	480	460
Glucose	mg/100 ml	16.8	14.6	13.3	13.8	12.7	13.4	12.9	13.1
Globulins	g/L	33	34	32	31	29	30	30	29
Albumin	g/L	28	29	26	26	23	22	24	23
Total protein	g/L	61	63	58	57	52	52	54	52

*These findings are representative of the results obtained from ten normolipidemic and ten hyperlipidemic animal experiments. There were no significant differences before and after treatment in any of these parameters.

TABLE IV. Quantitative Comparison of Blood Haematological Constituents Before and 2.5 h After Reinjection of Approximately 25% Delipidated Plasma Remixed With Original Blood Cells and Sham Treated Blood in a Normolipidemic and Hyperlipidemic Rooster*

		Normolipidemic rooster				Hyperlipidemic rooster			
		Lipid apheresis		Sham treatment		Lipid apheresis		Sham treatment	
Component	Dimension	Before	2.5 h after	Before	2.5 h after	Before	2.5 h after	Before	2.5 h after
White blood cells	$\times 10^9$/L	43.8	59.6	28.2	24.9	23.3	17.2	21.8	23.6
Red blood cells	$\times 10^{12}$/L	2.1	2.0	2.1	2.1	2.2	1.9	1.8	2.0
Haemoglobin	g/dl	10.0	9.7	9.9	9.8	9.7	8.2	9.3	9.2
Haematocrit	L/L	30.5	26.5	28.4	26.3	29.0	25.7	27.4	27.0
Mean corpuscular volume	fL	140.0	137.0	139.0	139.8	137.3	134.0	136.3	134.7
Mean corpuscular haemoglobin	pg	46.3	46.4	45.8	45.9	44.8	55.0	47.6	47.3
Mean corpuscular haemoglobin concentration	g/dl	33.0	34.0	32.8	33.2	32.7	40.9	33.7	34.9
Red cell distribution width		9.1	10.7	9.3	9.7	9.0	11.0	9.8	10.1
Platelet count	$\times 10^9$/L	3.4	4.5	4.2	4.5	4.8	6.7	4.9	4.7
Mean platelet volume	fl	3.5	3.9	3.7	3.8	3.9	4.1	3.9	3.9
Heterophils	%	30	35	36	31	36	42	32	36
Lymphocytes	%	56	46	51	53	53	37	54	49
Monocytes	%	10	16	9	12	7	18	10	11
Basophils	%	4	3	4	4	4	3	4	4

*These findings are representative of the results obtained from ten normolipidemic and ten hyperlipidemic animal experiments. There were no significant differences before and after treatment in any of these parameters.

vealed that only the lipid parameters were significantly different. This is in agreement with previously reported studies on human plasma [10].

Analyses of clinical chemical and haematological parameters indicated that the procedure was safe. The animals tolerated the treatment regimen well.

In these preliminary studies we have chosen the chicken as a model for the study of atherosclerosis research for a number of reasons. Firstly, the chicken is an omnivore, and normally ingests cholesterol-containing foods. This avoids the important objection which has been raised against the rabbit and other species in which cholesterol is essentially a foreign dietary substance. The chicken is easy to handle and is suitable for laboratory investigations. The chicken develops spontaneous atherosclerosis [43], and the chicken is capable of rapidly producing atherosclerosis after cholesterol feeding with hypercholesterolemia [44]; the plasma levels of cholesterol and triglyceride are similar to those in humans; the lipid composition of the lipoproteins resembles those of humans; somatic cells of chickens contain specific LDL receptors [45]; and finally there are many similarities between vascular lesions seen in chickens as a result of cholesterol diet and that of atherosclerosis observed in man [44].

The observations as provided by the present studies have led us to investigate a variety of larger animal models to determine whether LA may be a means of reversing atherosclerosis. Current indications suggest that rapid regression of atherosclerosis is possible in an animal model (Cham et al., in preparation). This may have important implications for the management of human atherosclerosis and indeed human clinical trials with LA as a possible means for regression of atherosclerosis have been approved and will soon start.

CONCLUSIONS

This paper describes a novel extracorporeal procedure, LA, which removes essentially all cholesterol and triglyceride from plasma while not affecting other important blood constituents. We report for the first time the use of LA on an animal model for the possible treatment of atherosclerosis. Several biochemical and haematological markers measured in blood of lipid apheresed animals demonstrated that LA was safe. Treated animals showed a marked, transient drop in plasma cholesterol and triglyceride concentrations indicating that other cholesterol pools were readily mobilised. These exciting observations suggest that LA may have a place in the treatment of atherosclerosis in the future and further research on both animals and humans is presently in progress.

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